

WHAT IS CLAIMED IS:

1. A method of phosphorylating a protein comprising contacting said protein with a soluble G1cNAc-phosphotransferase; and producing a phosphorylated protein.
- 5 2. The method of Claim 1, wherein said protein comprises an asparagine-linked oligosaccharide with a high mannose structure.
3. The method of Claim 1, wherein said soluble G1cNAc-phosphotransferase comprises the amino acid sequence in SEQ ID NO:2.
- 10 4. The method of Claim 1, wherein said soluble G1cNAc-phosphotransferase comprises an  $\alpha$  subunit, a  $\beta$  subunit and a site-specific proteolytic cleavage site interposed between said  $\alpha$  and  $\beta$  subunits, wherein said proteolytic cleavage site is not natural to said G1cNAc-phosphotransferase.
- 15 5. The method of Claim 4, wherein said  $\alpha$  subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3.
6. The method of Claim 4, wherein said  $\beta$ -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3.
- 20 7. The method of Claim 4, wherein said  $\alpha$ -subunit comprises amino acids 1-928 of SEQ ID NO:4.
8. The method of Claim 4, wherein said  $\beta$  subunit amino acids 1 to 328 of SEQ ID NO:5.

9. The method of Claim 4, wherein said soluble GlcNAc-phosphotransferase further comprises a  $\gamma$  subunit.
10. The method of Claim 9, wherein said  $\gamma$  subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6.
11. The method of Claim 9, wherein said  $\gamma$  subunit comprises the amino acid sequence of SEQ ID NO:7.
12. The method of Claim 1, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genease I proteolytic cleavage site.
13. The method of Claim 12, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.
14. The method of Claim 13, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:22.
15. The method of Claim 1, wherein said protein is a lysosomal hydrolase.
16. The method of Claim 15, wherein said lysosomal enzyme is selected from the group consisting of  $\alpha$ -glucosidase,  $\alpha$ -iduronidase,  $\beta$ -galactosidase A, arylsulfatase, N-acetylgalactosamine- $\alpha$  -sulfatase,  $\beta$ -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase,  $\beta$  -glucuronidase, Heparan N-sulfatase, N-Acetyl- $\alpha$ -glucosaminidase, Acetyl CoA- -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A

Cerebroside, Ganglioside, Acid  $\beta$ -galactosidase  $G_{M1}$  Galglioside, Acid -  
galactosidase, Hexosaminidase A, Hexosaminidase B,  $\alpha$ -fucosidase,  $\alpha$ -N-  
Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine  
amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase ,  
5 Sphingomyelinase, and Glucocerebrosidase  $\beta$ -Glucosidase.

17. The method of Claim 1, further comprising contacting said phosphorylated  
protein with an isolated phosphodiester  $\alpha$ -GlcNAcase.

18. The method of Claim 17, wherein said phosphodiester  $\alpha$ -GlcNAcase  
comprises the amino acid sequence of SEQ ID NO:18.

10 19. The method of Claim 17, wherein said phosphodiester  $\alpha$ -GlcNAcase is  
encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide  
sequence that hybridizes under stringent conditions to the complement of SEQ  
ID NO:17.

15 20. The method of Claim 1, wherein prior to said contacting the method  
comprises: culturing a host cell which comprises an isolated polynucleotide  
encoding soluble GlcNAc-phosphotransferase for a time under conditions  
suitable for expression of the soluble GlcNAc-phosphotransferase; and  
isolating said soluble GlcNAc-phosphotransferase.

20 21. The method of Claim 1, wherein prior to said contacting the method  
comprises culturing a host cell which comprises an isolated polynucleotide  
encoding soluble GlcNAc-phosphotransferase for a time under conditions  
suitable for expression of the soluble GlcNAc-phosphotransferase, wherein  
said soluble GlcNAc-phosphotransferase comprises an  $\alpha$  subunit, a  $\beta$  subunit

and a site-specific proteolytic cleavage site interposed between said  $\alpha$  and  $\beta$  subunits, wherein said proteolytic cleavage site is not endogenous to GlcNAc-phosphotransferase; isolating said soluble GlcNAc-phosphotransferase; cleaving said isolated soluble GlcNAc-phosphotransferase with a proteolytic enzyme specific for said proteolytic cleavage site; and mixing said  $\alpha$  and  $\beta$  subunits with a  $\gamma$  subunit of GlcNAc-phosphotransferase.

22. An isolated polypeptide comprising SEQ ID NO:2.
23. An isolated polynucleotide which encodes the polypeptide of Claim 22.
24. An isolated polynucleotide comprising SEQ ID NO: 1.
25. An isolated polynucleotide, which hybridizes under stringent conditions to the isolated polynucleotide SEQ ID NO:1 or the complement of SEQ ID NO:1.
26. An GlcNAc-phosphotransferase comprising an  $\alpha$  subunit, a  $\beta$  subunit and a site-specific proteolytic cleavage site interposed between said  $\alpha$  and  $\beta$  subunits, wherein said site-specific proteolytic cleavage site is not endogenous to GlcNAc-phosphotransferase.
27. An isolated polynucleotide, which encodes the GlcNAc-phosphotransferase of Claim 26.
28. The GlcNAc-phosphotransferase of Claim 26, wherein said  $\alpha$  subunit is encoded by nucleotides 165 to 2948 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 165 to 2948 of SEQ ID NO:3.

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29. The G1cNAc-phosphotransferase of Claim 26, wherein said  $\beta$ -subunit is encoded by nucleotides 2949 to 3932 of SEQ ID NO:3, or a sequence that hybridizes under stringent conditions to the complement of nucleotides 2949 to 3932 of SEQ ID NO:3.

5 30. The G1cNAc-phosphotransferase of Claim 30, wherein said  $\alpha$ -subunit comprises amino acids 1-928 of SEQ ID NO:4.

31. The G1cNAc-phosphotransferase of Claim 26, wherein said  $\beta$  subunit amino acids 1 to 328 of SEQ ID NO:5.

10 32. The G1cNAc-phosphotransferase of Claim 26, wherein said G1cNAc-phosphotransferase further comprises a  $\gamma$  subunit.

33. The G1cNAc-phosphotransferase of Claim 32, wherein said  $\gamma$  subunit is encoded by SEQ ID NO:6, or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6.

15 34. The G1cNAc-phosphotransferase of Claim 32, wherein said  $\gamma$  subunit comprises the amino acid sequence of SEQ ID NO:7.

35. The G1cNAc-phosphotransferase of Claim 26, wherein said site-specific proteolytic cleavage site is selected from the group consisting of a Furin proteolytic cleavage site, a Factor Xa proteolytic cleavage site, a Enterokinase proteolytic cleavage site, and a Genease I proteolytic cleavage site.

20 36. The G1cNAc-phosphotransferase of Claim 35, wherein said site-specific proteolytic cleavage site is a Furin proteolytic cleavage site.

37. The G1cNAc-phosphotransferase of Claim 36, wherein said Furin proteolytic cleavage site comprises SEQ ID NO:22.

38. A vector comprising the isolated polynucleotide of Claim 23.
39. A vector comprising the isolated polynucleotide of Claim 24.
40. A vector comprising the isolated polynucleotide of Claim 25.
41. A vector comprising the isolated polynucleotide of Claim 27.
- 5 42. A host cell comprising the isolated polynucleotide of Claim 23.
43. A host cell comprising the isolated polynucleotide of Claim 24.
44. A host cell comprising the isolated polynucleotide of Claim 25.
45. A host cell comprising the isolated polynucleotide of Claim 27.
46. A method of producing an  $\alpha$  and  $\beta$  subunit G1cNAc-phosphotransferase  
10 polyprotein comprising culturing the host cell of Claim 42 for a time and  
under conditions suitable for expression of the  $\alpha$  and  $\beta$  subunit G1cNAc-  
phosphotransferase polyprotein and collecting the  $\alpha$  and  $\beta$  subunit G1cNAc-  
phosphotransferase polyprotein produced.
47. . The method of Claim 46, wherein prior to said collecting, the  $\alpha$  and  $\beta$   
15 G1cNAc-phosphotransferase subunits are cleaved in the host cell by a site  
specific protease which is expressed in the cell, wherein said protease is  
specific for a protease cleavage site positioned between said  $\alpha$  and  $\beta$  subunits.
48. The method of Claim 46, further comprising after said collecting, the  $\alpha$  and  
20  $\beta$  subunits are cleaved with a protease specific for a protease cleavage site  
positioned between said  $\alpha$  and  $\beta$  subunits.
49. A method of producing an  $\alpha$  and  $\beta$  subunit G1cNAc-phosphotransferase  
polyprotein comprising culturing the host cell of Claim 45 for a time and  
under conditions suitable for expression of the  $\alpha$  and  $\beta$  subunit G1cNAc-

phosphotransferase polyprotein and collecting the  $\alpha$  and  $\beta$  subunit GlcNAc-phosphotransferase polyprotein produced.

50. . The method of Claim 49, wherein prior to said collecting, the  $\alpha$  and  $\beta$  GlcNAc-phosphotransferase subunits are cleaved in the host cell by a site specific protease which is expressed in the cell, wherein said protease is specific for a protease cleavage site positioned between said  $\alpha$  and  $\beta$  subunits.

51. The method of Claim 49, further comprising after said collecting, the  $\alpha$  and  $\beta$  subunits are cleaved with a protease specific for a protease cleavage site positioned between said  $\alpha$  and  $\beta$  subunits.

52. A phosphorylated protein obtained by the method of Claim 1.

53. A phosphorylated protein obtained by the method of Claim 17.

54. A method of treating a patient suffering from a lysosomal storage disease comprising contacting a lysosomal hydrolase with the GlcNAc-phosphotransferase of Claim 26 to produce a lysosomal hydrolase with an N-acetylglucosamine-1-phosphate; removing said N-acetylglucosamine by contacting said lysosomal hydrolase with a phosphodiester  $\alpha$ -GlcNAcase to produce a phosphorylated lysosomal hydrolase isolating said phosphorylated lysosomal hydrolase; and administering an amount sufficient to treat said disease the isolated phosphorylated lysosomal hydrolase.

55. A method of treating a patient suffering from a lysosomal storage disease comprising contacting a lysosomal hydrolase with the GlcNAc-phosphotransferase of Claim 32 to produce a lysosomal hydrolase with an N-acetylglucosamine-1-phosphate; removing said N-acetylglucosamine by

contacting said lysosomal hydrolase with a phosphodiester  $\alpha$ -GlcNAcase to produce a phosphorylated lysosomal hydrolase isolating said phosphorylated lysosomal hydrolase; and administering an amount sufficient to treat said disease the isolated phosphorylated lysosomal hydrolase.

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